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MATCHING WATERSHED AND OTOLITH CHEMISTRY TO ESTABLISH NATAL ORIGIN OF AN
ENDANGERED DESERT LAKE SUCKER

by

Deanna Strohm

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

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Logan, Utah

2015

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ABSTRACT

Matching Watershed and Otolith Microchemistry to Establish Natal Origins of an
Endangered Desert Lake Sucker

by

Deanna Strohm, Master of Science

Utah State University, 2015

Major Professor: Phaedra Budy
Department: Watershed Science

Like many native endemic desert freshwater fish species, the June Sucker (*Chasmistes liorus*) is currently listed as endangered. Implicit within the June Sucker recovery plan, is that spawning habitat restoration must result in natural recruitment. I used otolith microchemistry to establish natal origins of the potamodromous June Sucker endemic to Utah Lake, UT, USA, ultimately in order to evaluate whether tributary habitat restoration results in natural recruitment. My specific objectives included; 1) quantify and characterize the extent of chemical variation among the three main spawning tributaries; 2) determine the relationship between otolith microchemistry and tributary chemistry; and 3) develop and validate a classification model to identify stream origin. I quantified the molar ratios Sr:Ca, Ba:Ca, and Mg:Ca for water and otolith chemistry from the three main tributaries to Utah Lake, UT during the summer of 2013.

Water chemistry differed significantly among all three spawning tributaries (Sr:Ca P-value <0.05; Ba:Ca <0.05; Mg:Ca P-value <0.05); Ba:Ca and Sr:Ca were identified as the most important variables driving the classification models. I observed a strong linear relationship between water and otolith microchemistry for Sr:Ca and Ba:Ca, but not for Mg:Ca ($R^2 = 0.77$ P-value <.05; $R^2 = 0.83$ P-value <.05; $R^2 = .0017$, P-value= 0.71, respectively). Based on classification models of otolith element:Ca signatures, I was able to accurately classify individual fish to their natal tributary (classification tree 89% accuracy; random forest model 91% accuracy), and was able to determine if the fish's origin is wild vs. hatchery with 100% accuracy. The use of classification trees and random forest for classification analyses may provide a more powerful method for classification in studies using otolith microchemistry. Overall, this study will aid in evaluating the effectiveness of restoration, track progress toward recovery, help prioritize future restoration plans for the June Sucker in Utah Lake, and can be applied to other imperiled system with species of conservation concern.

(59 pages)

PUBLIC ABSTRACT

Matching Watershed and Otolith Microchemistry to Establish Natal Origins of an
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by

Deanna Strohm, Master of Science

Utah State University, 2015

Major Professor: Phaedra Budy
Department: Watershed Science

Like many native endemic desert freshwater fish species, the June Sucker (*Chasmistes liorus*) is currently listed as endangered. Managers have increasingly turned to habitat restoration as a key component to recovery plans. For endangered species, one of the primary outcomes of habitat restoration is that it should result in successful reproduction and recruitment of individuals into the adult population. Confirmation of natural recruitment as a function of habitat restoration can only be achieved by establishing natal origins.

Recent research has proven the validity of otolith microchemistry, a technique that analyzes small quantities of elements, to trace potamodromous fish to their natal tributaries. Previous studies have documented that localized habitats in terms of microchemistry are reflected in otolith composition, thereby potentially making this a

valuable way of determining fish origins. The primary goal of this study is to use otolith microchemistry to establish natal origins of June Sucker, ultimately in order to evaluate whether tributary habitat restoration results in natural recruitment. To accomplish this I first determined if the water chemistry among the three main spawning tributaries differed from one another. Second, I determined if the otolith chemistry reflected the otolith chemistry. Lastly, I developed a statistical model capable of classifying fish to their respective tributary based the element:calcium ratios in the otolith microchemistry.

Water chemistry differed significantly among all three spawning tributaries, and I observed a strong and significant relationship between otolith chemistry and water chemistry. The classification models based on otolith element:Ca signatures were capable of accurately classifying individual fish to their natal tributary (classification tree 89% accuracy; random forest model 91% accuracy), increasing the ability to determine if the fish's origin is wild vs. hatchery. The data obtained from this study will advance the current understanding of the June sucker recruitment dynamics and result in a fundamental improvement in our ability to determine where natural recruitment into the adult spawning population is occurring. In addition, this knowledge may help evaluate factors limiting recruitment in Utah Lake tributaries, identify future restoration localities, and assist effectiveness monitoring of spawning habitat restoration efforts.

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INTRODUCTION

Anthropogenic alterations to lotic freshwaters including stream fragmentation, sediment loading, introduced non-native species, and revised flow regimes, are linked to alarming rates of decline in freshwater fauna throughout the nation (Ricciardi and Rasmussen 1999; Jelks et al. 2008). Freshwater fish, in particular, have experienced one of the fastest rates of decline of all species globally (Ricciardi and Rasmussen 1999). Since 1900, there have been 57 extinctions observed in freshwater fish species in North America alone (Miller et al. 1989, Jelks et al. 2008). Alterations in flow regimes for agricultural and urban uses are a primary cause of habitat degradation, by reducing seasonal base flows and modifying the duration, frequency, and magnitude of high flow events (Budy et al. 2015). Changes in flow regimes influence the physical, biological, and abiotic conditions of the stream by altering riparian vegetation, channel morphology, habitat diversity, and temperature and dissolved oxygen patterns (Stromberg et al. 2005; Budy et al. 2015). Current rates of extinction for freshwater fishes are roughly 960 times greater than historic background rates from fossil record, likely due to these anthropogenic alterations (May et. al 1995; Pimm et al. 1995; Burkhead 2012).

Freshwater species in the arid, western United States have proven exceptionally vulnerable to human disturbance. This vulnerability is due, in part to a high amount of endemism and competition with humans for water resources; thus, a significant proportion of species have already gone extinct and many desert fish species are currently listed as threatened or endangered (Minckley and Douglas 1991; Burr and Mayden 1992; Roni et al.

2002). In addition, many non-game species are perceived to lack charisma and have no economic value, and therefore, have been understudied (McKinney 1999, Budy et al. 2015). As a result, large knowledge gaps remain about the natural history and ecology of early life stages of many endemic western fishes further hindering our conservation success (Gadomski et al. 2001; Cooperman and Markle 2003). Furthermore, specific evolutionary life histories driving resource and habitat use (e.g., potamodromy) can further complicate their management and conservation (Olden et al. 2008; Laub et al. 2015).

Among the arid freshwater systems in the western United States, desert lakes are highly dynamic ecosystems, as they experience a wide range of natural disturbances such as drought, erosion, and extreme temperature and water level fluctuations (Scheffer 2001; Buelow 2006). The fishes that persist in these volatile conditions often express highly specialized suite of life history traits including; large body size, long life span, delayed maturity, and specialized feeding behaviors (Gaston 1994; Olden et al. 2008). Species with these biological life history traits are often more vulnerable to long-term changes in the environment and have a higher risk of extinction (Olden et al. 2008). In addition, some desert lake fish species are potamodromous, demonstrating migrations from a lake habitat into tributary habitats to spawn. Fishes expressing potamodromy are vulnerable to stream habitat degradation, because they require the use of tributaries for spawning and early life stages (Santos et al. 2011). These tributaries are often exposed to significant water development and regulation, which often results in extreme degradation to spawning and rearing habitat. Reduced flows during critical spawning

and rearing months, limit suitable spawning habitat access, reduce habitat diversity, and decrease connectivity to rearing habitat for the June Sucker. As stream habitat restoration has necessarily become a key component to many recovery efforts for endangered fish species, there is a need for tools to aid managers in more rapidly evaluating critical areas for conservation.

The primary goal of localized stream habitat restoration is to reverse physical damage and increase habitat heterogeneity, and in-turn, induce a favorable ecological response (Palmer et al. 1997; Bond and Lake 2003). Habitat restoration approaches such as, channel and streambed reconfiguration, placement of in-stream structures, and riparian vegetation establishment, often focus on critical spawning and nursery habitat for early life stages (Kauffman et al. 1997). During the early life stages of development, fishes naturally experience the highest rates of mortality; consequently, the early life stages can be one of the most critical time periods determining population success (Schiemer et al. 2002). However, identifying optimal locations for restoration of critical spawning and nursery habitat, and assessing the effectiveness of habitat manipulations in restoring ecosystem structure and function is rarely properly evaluated often due to time, effort, and financial constraints (Bond and Lake 2003; Budy and Schaller 2007).

One of the most critical components to recovery of an endangered fish is that reproduction results in successful recruitment of young into the adult spawning population (NCR 1995; Budy and Schaller 2007). As such, improving natural recruitment is often the

primary justification for habitat restoration efforts (Budy and Schaller 2007); however, it is often challenging to determine if habitat restoration actually results in natural recruitment. One way to determine if habitat restoration enhances natural recruitment is to determine the natal origin of newly recruited individuals. In other words, are fish successfully spawning in areas of better habitat quality and/or areas of restoration focus? Knowing the localities where fish reproduction has been successful can allow managers to evaluate factors that both limit and promote recruitment and track progress toward recovery. Otolith microchemistry has recently become a useful tool to determine the natal origin and track movement of fish in marine environments, and is increasingly being used in freshwater systems (Elsdon et al. 2008). This technique could be used to determine natal origin and thus aid in evaluating habitat restoration effectiveness by determining if areas of habitat restoration are successful in enhancing natural recruitment.

Recent research has proven the validity of otolith microchemistry, a technique reliant on measuring the elemental chemistry in otolith calcium carbonate, to trace potamodromous fish to their natal tributaries, as well as, answer a variety of pressing ecological questions (Elsdon et al. 2008). Otoliths accrete new calcium carbonate (aragonite) and protein onto the surface daily (Campana 1999, Elsdon et al. 2008), and along with daily accretions of aragonite, trace elements with a similar charge to calcium are substituted for calcium during crystallization (Bickford and Hannigan 2005). Trace elements are incorporated into the crystalline matrix in proportion to their abundance in the ambient water (Elsdon et al. 2008; Pangle et al. 2010).

Otoliths are acellular and metabolically inert, so any element substituted for Ca onto the growing surface is permanently retained.

When a fish experiences changes in its chemical environment, those changes are recorded onto the surface of the otolith (Campana 1999), a record of the fish's environmental experiences. The core of the otolith represents the origin of its birth, and the outer-most ring of the otolith represents its most recent life experiences to the time of death. In order to track fish movement using otolith microchemistry, there must be significant differences in water chemistry among the water bodies of interest, they must be temporally stable, and the differences in water chemistry must be reflected in the otolith chemistry. Previous studies have documented that localized habitats, are reflected in otolith composition in terms of microchemistry, thereby potentially making this a valuable way of determining fish origins (e.g., Wells et al. 2003; Bickford and Hannigan 2005; Pangle et al. 2010).

The June Sucker (*Chasmistes liorus*), is currently listed as endangered, and numerous management actions, including tributary habitat restoration, have been implemented in hopes to recover this highly imperiled species. Before European settlement in 1849 June Suckers were historically abundant in Utah Lake (Janetski 1990); however, in 1997 there were estimates of as few as 300 wild spawning individuals in the population (USFWS 1999; Cooke et al. 2005). When listed as endangered in 1986, little to no evidence existed to indicate successful June Sucker reproduction in the wild. The few spawning adults caught were primarily spawning in

the Provo River (Radant 1986), with little knowledge of reproductive activity in the other five historical spawning tributaries.

Based on preliminary analysis of water chemistry, I hypothesized that otolith microchemistry could be used to determine the natal origin of the endangered June sucker, in order to address one of the current knowledge gaps of June Sucker recruitment. My specific objectives were to: 1) quantifying and characterize the extent of chemical variation among the three main spawning tributaries; 2) understand the relationship between June Sucker otolith microchemistry and tributary chemistry and; 3) develop and validate a model to identify stream origin using otolith microchemistry data. The data obtained from this study will advance the current understanding of June Sucker recruitment dynamics in Utah Lake and its three main tributaries, by improving our ability to determine the occurrence and location of natural recruitment into the adult spawning population. In addition, the information gained from this study has implications for other imperiled potamodromous species of conservation concern.

METHODS

Study site and organism

The endemic June Sucker (*Chasmistes liorus*) is potamodromous, demonstrating migrations from a lake habitat, Utah Lake, UT, into now degraded tributary habitats to spawn (Figure 1). Over the past twenty-five years, management efforts have included captive rearing and augmentation, common carp (*Cyprinus carpio*) removal, revised tributary flow regimes, and

spawning habitat restoration projects. Hobble Creek underwent a delta restoration project in 2008, designed to relocate the channel and reconnect it to Utah Lake to promote June sucker spawning, rearing, and recruitment (USDI 2008). A similar delta restoration project for Provo River is currently being planned. While significant efforts have been made to conserve the species, it is unknown if sufficient natural recruitment into the population is occurring.

Utah Lake is a remnant of Pleistocene Lake Bonneville (Buelow 2006), and as one of the largest natural freshwater lakes west of the Mississippi, it has a surface area of approximately 388 km² (Figure 1). Utah Lake has a relatively small volume compared to its large surface area, due to a relatively shallow depth, with an average depth of 2.9 meters and a maximum depth of 4.2 meters. Utah Lake is fed by three major tributaries: Provo River, Hobble Creek, Spanish Fork; and three minor tributaries: American Fork, Battle Creek, and Spring Creek (Figure 1). June Sucker have been anecdotally documented spawning in all six of these tributaries in recent years, with a notably large proportion of fish spawning in the Provo River compared to the other tributaries. However; the historic flow regimes and morphologic features of these tributaries have been dramatically altered for urban and agricultural purposes. In addition, the native food web of Utah Lake has been dramatically altered. Historically, the Utah Lake food web consisted of 14 native fish species; however, today almost all of native species have been extirpated, one species is extinct, and the June sucker is listed as endangered. The June Sucker and the Utah Sucker (*Catostomus ardens*) are the only native species in the lake, and the 16 non-native introduced species in the Utah Lake are all potential competitors and predators of

the June Sucker. In addition, common carp make up $> 80\%$ of the lakes biomass and have been in part, responsible for an ecosystem state change, from a vegetated, clear lake, to a turbid, eutrophic lake. Alterations to tributary flow regimes, nonnative introductions, in addition to spawning and nursery habitat loss, all pose a significant threat to the survival and recovery of the June Sucker.

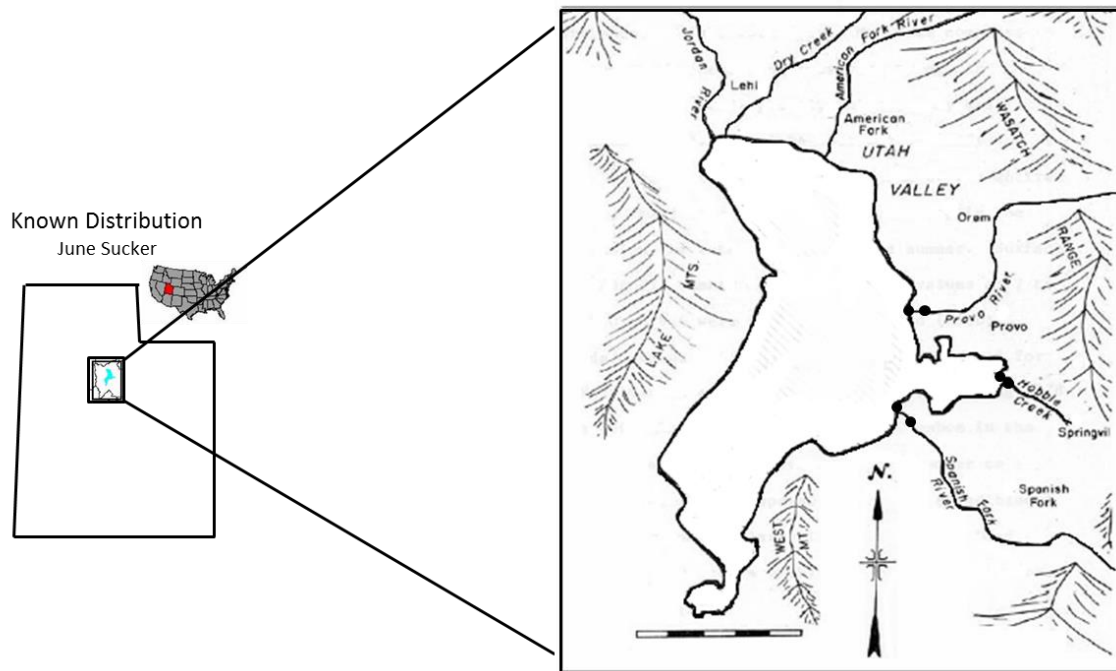


Figure 1. The location of Utah Lake and its major tributaries; Provo River, Hobbie Creek, and Spanish Fork, shown within the state of Utah. The dots indicate the upstream and downstream study site locations where monthly water chemistry was collected and the fish cages incubated during the summer of 2013. Picture modified with permission from Buelow 2006

Quantifying the extent of chemical variation among spawning tributaries

To determine the extent of chemical variation in water chemistry among tributaries, I collected water samples within each of the three major tributaries; Provo River, Hobble Creek and Spanish Fork. The water samples were taken at documented spawning sites to represent the area where egg development and initial elemental accretion will begin. I collected water samples each month throughout the summer (June - end of August 2013) to detect intra-annual variation in major and trace element chemistry that might occur. I collected two 60 milliliter water samples from an upstream and downstream site within each tributary separated by one to three kilometers in distance. The stream water was filtered through 0.45 μm sterilized nylon membrane filter and placed in clean plastic Nalgene® containers. I acidified the samples on site with concentrated nitric acid to a concentration of 1% of the 60 ml sample for preservation up to six months. The water samples were analyzed at Utah State University Water Research Laboratories using inductively coupled plasma mass spectrometry (ICP-MS). In addition, I collected water quality data at time of sampling, including: temperature, pH, conductivity, salinity, total dissolved solids, and dissolved oxygen with an YSI Professional Plus Series multiparameter meter.

Experimental identification of the unique chemical signature of the tributaries on otolith microchemistry

To determine if there was adequate variation in otolith microchemistry to discriminate among the different tributaries, I conducted an *in situ* natural cage experiment. I constructed cages out of 6 inch diameter PVC pipe; and stocked the cages at the upstream and downstream site in each tributary with month old June Suckers obtained from the Utah Division of Wildlife Resources, Fisheries Experimental Station (FES). I measured a subsample of fish for total length to the nearest mm before being placed in the cages. I placed three replicate cages at the upstream location and an additional three were placed at the downstream location (Figure 1). I placed the downstream cages near the mouth of the stream at the lake interface to allow for comparison between lake influences on tributary signals. The upstream cages remained at the spawning location to incorporate the natal signature during egg incubation and swim-up. I stocked the cages with ten to fifteen fish per cage for a total of six cages per stream resulting in approximately sixty to ninety fish per tributary. To develop a strong elemental otolith signature for each tributary, the cages remained in the stream from July 17th, 2013 to August 30th, 2013. All of the cages and fish were lost at the upstream site of the Spanish Fork due to a high water event, and two cages were lost on the Provo River upstream site due to vandalism.

At the end of the experiment I collected fish from cages, euthanized them, measured total length to the nearest 1 mm (Table A.1), and immediately transferred the fish into clean glass shell vials and placed them on ice until they were able to be frozen. Whole fish were kept

frozen until otoliths were extracted. I extracted the lapilli and sagittal otoliths under a dissecting microscope with cleaned tools that were non-metallic, or otherwise wrapped in Teflon. Once the otolith was out of the endolymph sac, I cleaned it of remaining tissue and rinsed with deionized water. The cleaned otoliths were then adhered to glass slides with double-sided tape. Mounted otoliths were taken to Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility, where I analyzed them for a suite of trace elements using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS).

Otolith microchemistry

I used the Finnegan Element MAT 2 high-resolution inductively coupled plasma mass spectrometer, equipped with a new Wave 193-nm laser ablation system to analyze otolith microchemistry. Sagittal and lapilli otoliths from fish were analyzed for a suite of elements including Ca, Ba, Mg, Mn, Sr, Pb and U. However, initial screening of the data revealed sagittal otoliths had higher and more consistent concentrations of elements and thus were used for all further analyses. To examine the most recent period of the fish's life, representing the time in the stream, I ablated a 50 μm pathway around half of the outer perimeter of the otolith with the laser. I used raw (e.g., unsectioned, unpolished) otoliths and measured otolith element concentrations along the outer edge (vs. a transect across the otolith) of the otolith, in order to capture the most recent time of the fish's life, i.e. the time spent in the tributary (Pangle et al. 2010).

To correct for machine drift, background noise, and normalize the data; a gas blank, FEBS-1 otolith standard, and NIES-022 standard were analyzed before and after every tenth sample in the ablation sequence. I corrected the raw data output for background noise by subtracting the average blank values from the average intensity for each element concentration. I then normalized the background corrected data to the FEBS-1 calcium standard according to the following equation (1):

$$(1) \quad \text{element:Ca} = \frac{I_{\text{element sample}}}{I_{\text{Ca sample}}} \times \left[\frac{C_{\text{element standard}}}{C_{\text{Ca standard}}} \times \frac{I_{\text{Ca Sample standard}}}{I_{\text{element sample Standard}}} \right]$$

where I_{sample} is the element intensity from the ablation (cps), $I_{\text{sample standard}}$ is the element NRC standard average from each sequence run, and C is the average NRC otolith standard concentration (ppm) from the sequence (Lee C-T A. 2006). For an element to be included in the analysis, it had to be above the limit of detection (LOD), and it had to be measured precisely (Pangle et al. 2010). To meet the LOD, the element had to be greater than three standard deviations of the average background levels for that element. Manganese, Pb, and U failed to meet the limit of detection and were removed from further analysis. I calculated the relative standard deviation (RSD) for each element, as the standard deviation/mean x 100; which serves as measurement for precision (Hand et al. 2008). A low RSD value indicates low variability of the data and greater precision. The lower limit for precision was set at 10.5%, if the RSD was above 10.5% it was considered to not be measured precisely and it was removed from the data set (Hand et al. 2008). I reported the mean element:Ca values in parts per million (ppm) for the

otolith edge (Table A.1). In addition, I calculated the relative concentration at which elements were incorporated into the otolith with respect to their occurrence in the ambient water as the partition coefficient; where the partition coefficient = $(\text{element:Ca})_{\text{otolith}} / (\text{element:Ca})_{\text{water}}$ (Campana 1999).

Statistical analysis

I first performed descriptive statistics to assess normality and homoscedasticity for otolith and water element:Ca data. All of the data were log transformed prior to statistical analysis, which slightly improved normality and heteroscedasticity. To quantify the extent of chemical variation among spawning tributaries, and to determine if there was evidence of intra-annual variation in water chemistry, I performed Kruskal-Wallis tests on the water chemistry data. The Kruskal-Wallis was chosen over the ANOVA because the water chemistry data are not normally distributed. The Kruskal-Wallis is a non-parametric; therefore, does not assume normal distribution; it assigns a rank to each observation in the data-set, to test the null-hypothesis that the mean ranks of the groups are the same (McDonald 2014). To identify the unique chemical signature of the tributaries, I developed two classification models, a classification tree model and a random forest model, both for the water chemistry data (Cutler et al. 2007). To explore the relationship between otolith chemistry and tributary chemistry, I first ran a linear regression; with the individual fish otolith element:Ca ratios as a function of the water chemistry element:Ca ratios. For the water chemistry parameter in the linear

regression, I chose the mean element:Ca ratio from the water chemistry data with date closest to fish removal (August 15th 2013, 15 days prior to fish death). Although this approach required some level of pseudo-replication (only one water sample per site), it provided a helpful preliminary graphical representation of relationship between water and otolith chemistry. In addition, I calculated the Pearson correlation coefficients to determine if there was a linear relationship between the uptake of Sr, Ba, and Mg into the otolith (the partition coefficient) and water quality variables (temperature, dissolved oxygen, salinity, and pH).

Next, I used two classification models, classification tree analysis and random forest model, to determine if there was adequate variation in otolith element:Ca concentrations to discriminate among tributaries in order to classify individual fish to their respective tributaries. I performed all analyses in R freeware software version 2.15.1 (R Development Core Team 2012) in packages MASS 7.3-18, verification 1.37, randomForest 4.6-7, rpart 4.1-8. I cross-validated the models using a ten-fold jack-knife technique, which tests the accuracy and predictive capabilities of the classification models. Lastly, I performed non-metric multidimensional (NMDS) scaling ordination in R, package vegan, to visually demonstrate the otolith microchemistry patterns among fish from different tributaries observed in the classification tree and random forest model. I did not log transform the data for the NMDS analysis.

RESULTS

Water chemistry did not significantly vary temporally within any of the tributaries for from the time period of June through the end of August. Water chemistry differed significantly among all three spawning tributaries for Log_e transformed Sr:Ca (Kruskal Wallis: $X^2=16.9$; $df=2$; P-value <0.001); Ba:Ca Ca (Kruskal Wallis: $X^2=8.4$; $df=2$; P-value 0.015); and Mg:Ca (Kruskal Wallis: $X^2=15.1$; $df=2$; P-value <0.001; Figure 2); however, there was no significant intra-annual difference in water chemistry over the duration of the study (Table 1). Tributary water chemistry could be accurately classified based on the element:Ca ratio; classification models demonstrated 100% classification accuracy for the classification tree analysis and random forest (Figure 3). The Sr:Ca and Mg:Ca were identified as the most important variables driving the classification in both models, as indicated by the random forest variable importance plot, a variable selection method, and the classification tree (Figure 3).

Otolith microchemistry was strongly associated with the water chemistry of the respective tributaries. I observed a strong and significant linear relationship between otolith and water chemistry for Sr:Ca ($r^2 = 0.77$; P-value <.05) and Ba:Ca ($r^2 = 0.83$, P-value <.05), but not for Mg:Ca ($r^2 = .0017$, P-value= 0.71) (Figure 4). The partition coefficients differed among elements and were calculated for the Sr:Ca ratio as 0.39 (N=80; SE=0.004); 0.08 for Ba:Ca (N=80; SE=0.08); and 9.4×10^{-5} for the Mg:Ca (N=80; SE= 4.6×10^{-6}); a value of one would indicate there is no elemental discrimination (Campana 1999). The Pearson correlation

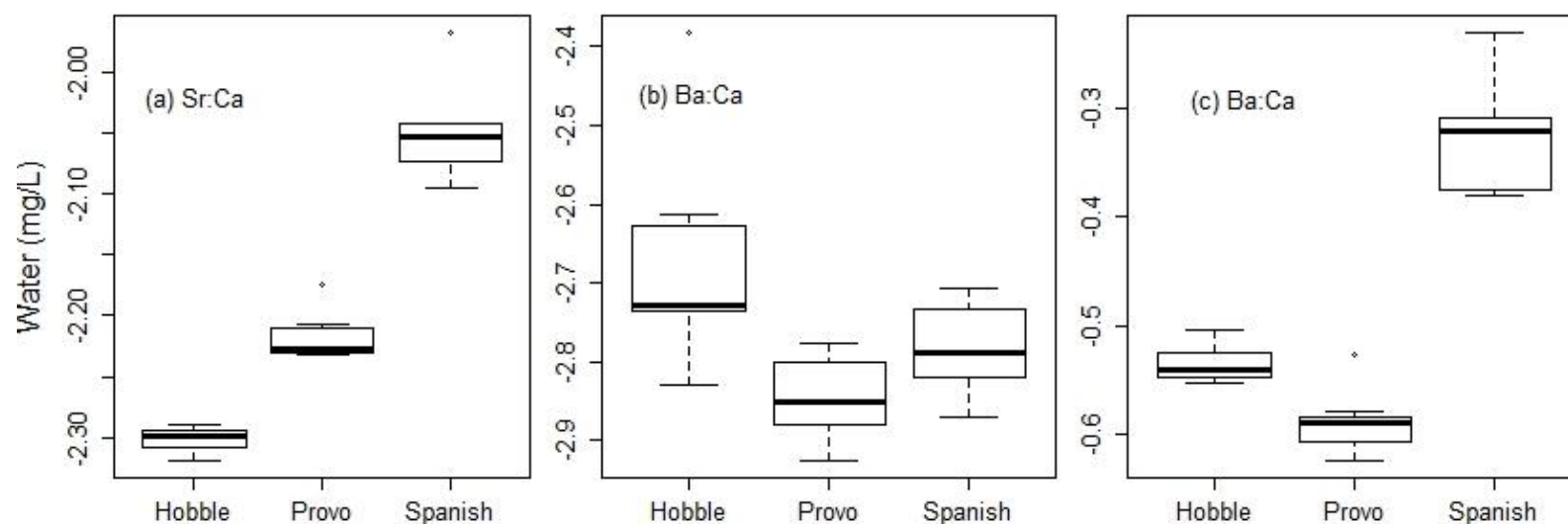


Figure 2. Boxplots of log transformed element:Ca ratio of water chemistry from each tributary for each element (Sr:Ca; Ba:Ca; and Mg:Ca). Horizontal lines in the boxes represent the median, the top and bottom of the box represent the first and third quantile. Water samples were collected monthly (June through end of August 2013) at the upstream and downstream study site locations within Hobble Creek, Provo River, and Spanish Fork.

Table 1. Non-parametric Kruskal-Wallis test, showing the mean ranks of element:Ca ratio (ppm) for water chemistry are the not significantly different across sampling months (June through end of August 2013) from the three main June Sucker spawning tributaries of Utah Lake, Utah.

Kruskal Wallis				
Stream	Element	Chi squared	df	P-value
Provo River	Sr:Ca	0.32	3	0.96
	Ba:Ca	5.7	3	0.13
	Mg:Ca	1.9	3	0.59
Hobble Creek	Sr:Ca	2.1	3	0.54
	Ba:Ca	5.7	3	0.13
	Mg:Ca	5.3	3	0.15
Spanish Fork	Sr:Ca	1.4	3	0.7
	Ba:Ca	4.7	3	0.19
	Mg:Ca	1.4	3	0.7

coefficients indicated there was a linear relationship between the Ba partition and temperature (Pearson's correlation coefficient= 0.70). However, there was severe heteroscedasticity in the standardized residuals and the data suggested an exponential model, which fit better than the linear model. The exponential model with temperature, explained a high amount of variation in Ba partition coefficient ($R^2=0.75$; Figure A.1). However the model assumption of homoscedasticity was violated due to the high variation in Ba at low temperatures. The exponential model indicates that the Ba partition coefficient exponentially decreases as temperature increases as temperature increases; the barium decreases exponentially. There were no strong linear relationships for the Sr partition coefficient and any of the abiotic variables.

In general, the classification models performed similarly, both exhibiting relatively high overall model classification accuracy, and both exhibiting some classification error associated with two of the streams. The random forest model

demonstrated an overall classification accuracy of 91.4%, correctly classifying 83.3% of the FES hatchery fish, 100% of the Hobble Creek fish, 88.5% of the Provo River fish, and 70% of the Spanish Fork fish. This model exhibited the most error associated with the Provo River and Spanish Fork River; three fish from Spanish Fork were misclassified as Provo River fish, and three Provo River fish were misclassified as Spanish Fork fish (Table 2). The classification tree showed similar results, with an overall classification rate of

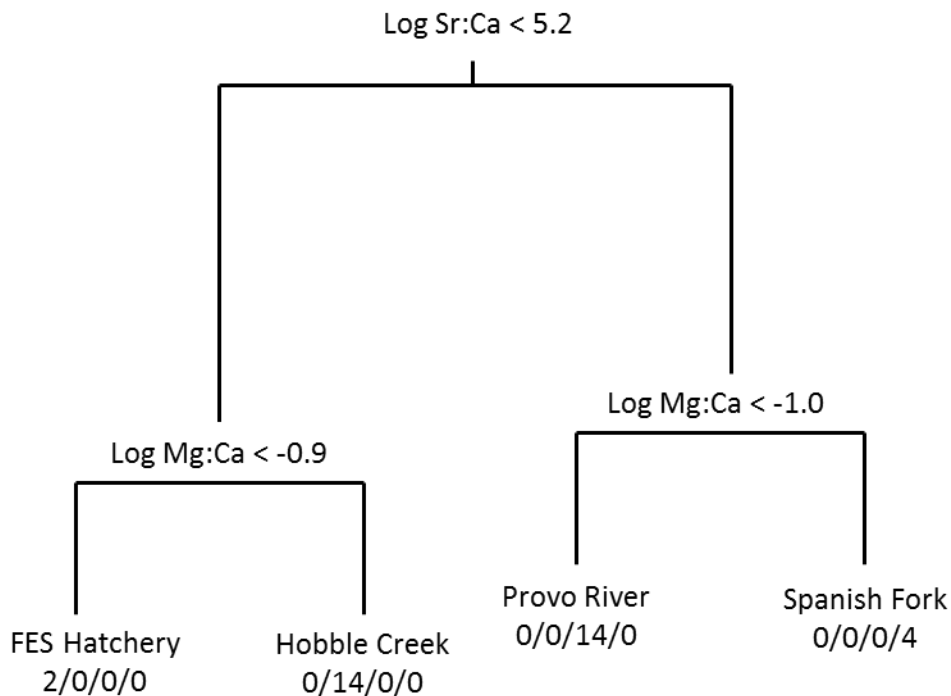


Figure 3. Classification tree for the tributary and FES hatchery water chemistry data. The data were the average element:Ca ratios (Sr:Ca, Ba:Ca, and Mg:Ca) collected from June through August 2013, for the upstream and downstream sampling locations within each tributary. Each split in the tree is a condition for the classification. The slashes at the nodes represent each tributary (FES Hatchery/Hobble Creek/Provo River/Spanish Fork), and the numbers represent how many individual samples are classified to that tributary. The classification tree model is capable of predicting streams based on log element:Ca ratio with 100 % accuracy.

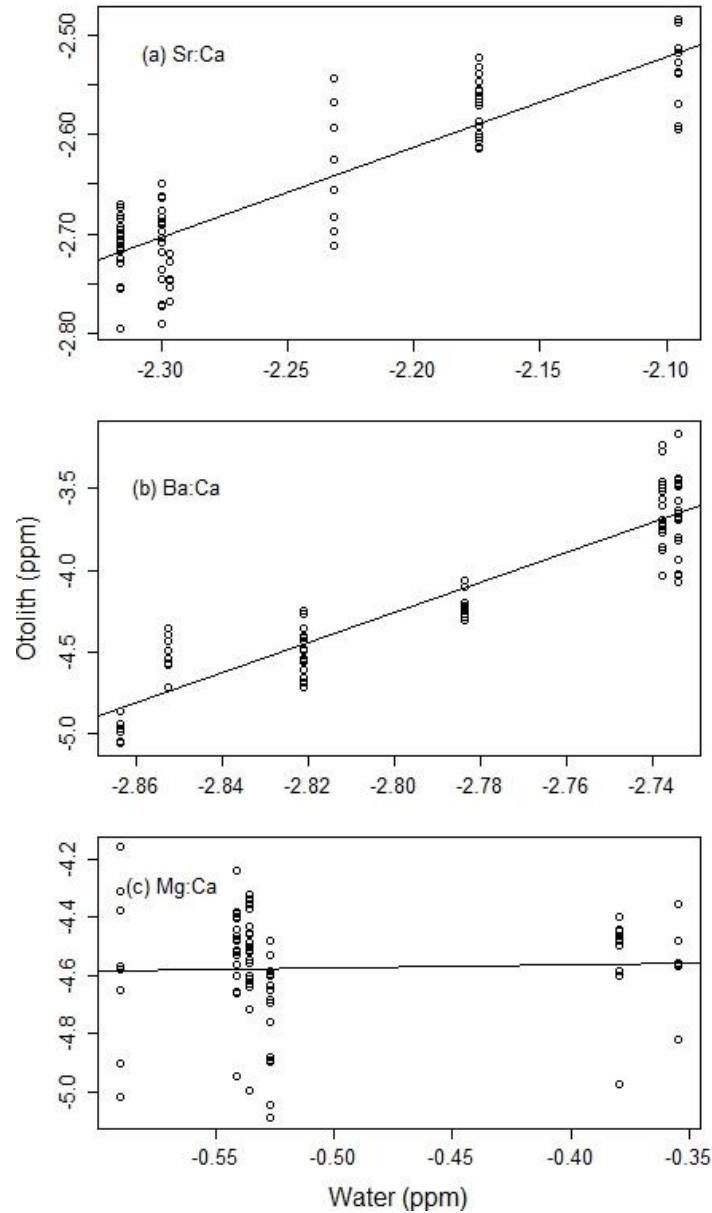


Figure 4. (a) Linear regression of Sr:Ca June Sucker sagittal otolith chemistry against Sr:Ca water chemistry for the three tributaries and FES hatchery. ($P < 0.001$, $R^2=0.77$, $n=78$). (b) Linear regression of Ba:Ca June Sucker sagittal otolith chemistry against Ba:Ca water chemistry for the three tributaries and hatchery. ($P < 0.001$, $R^2=0.84$, $n=78$). (c) Linear regression of Mg:Ca June Sucker sagittal otolith chemistry against Mg:Ca water chemistry for the three tributaries and hatchery ($P < 0.001$, $R^2=0.0017$, $n=78$).

88.9%, correctly classifying 100% of the FES hatchery fish, 94.8% of the Hobble Creek fish, 84.6% of the Provo River fish, and 70% of the Spanish Fork fish. Similar to the random forest model, three fish from the Provo River were misclassified as Spanish fork fish, and three fish from the Spanish Fork were misclassified as Provo River fish as indicated by Table 3.

The most important variables driving the classification models were Ba:Ca and Sr:Ca ratios in the otoliths. The random forest variable importance plot, and the fitted classification tree, both identified Ba:Ca and Sr:Ca as the most important elements driving the model respectively (Figure 5 and 6). Hobble Creek has the highest Ba:Ca ratio among all the tributaries and was the first condition identified in the classification tree, followed by further splits in the Sr:Ca ratios. The ‘important variable selection’ procedure for the random forest indicated otolith Mg:Ca ratio was the least important variable in the classification model for the otolith chemistry (Figure 5); this pattern was also observed in the classification tree model (Figure 6). The NMDS ordination clearly demonstrates the separation and overlaps in otolith chemistry among tributaries (Figure 7); FES hatchery and Hobble Creek demonstrate complete separation, and conversely otolith chemistries from Spanish Fork and Provo River demonstrated the partial overlap.

DISCUSSION

In order to evaluate whether or not habitat restoration results in or contributes to natural recruitment, it is imperative to establish the natal origin (e.g., location;

Table 2. Out of bag confusion matrix for the random forest classification model based on 2013 June Sucker sagittal otolith chemistry from the FES hatchery and three tributaries; Hobble Creek, Provo River, and Spanish Fork, with the upstream and downstream locations pooled for each stream. The columns represent the stream the fish were classified to. The rows represent the streams the fish came from. The last column is the percent of fish correctly classified to their respective stream (PCC).

Stream	FES Hatchery	Hobble Creek	Provo River	Spanish Fork	PCC
FES Hatchery	5	0	1	0	83.3
Hobble Creek	0	39	0	0	100
Provo River	0	0	23	3	88.5
Spanish Fork	0	0	3	7	70

Table 3. The ten-fold cross validated confusion matrix for the classification tree model based on 2013 June Sucker sagittal otolith chemistry from the FES hatchery and three tributaries; Hobble Creek, Provo River, and Spanish Fork, with the upstream and downstream locations pooled for each stream. The columns represent the stream to which the fish were classified. The rows represent the streams the fish came from. The last column is the percent of fish correctly classified to their respective stream (PCC).

Stream	FES Hatchery	Hobble Creek	Provo River	Spanish Fork	PCC
FES Hatchery	6	0	0	0	100
Hobble Creek	0	37	0	2	94.8
Provo River	1	0	22	3	84.6
Spanish Fork	0	0	3	7	70

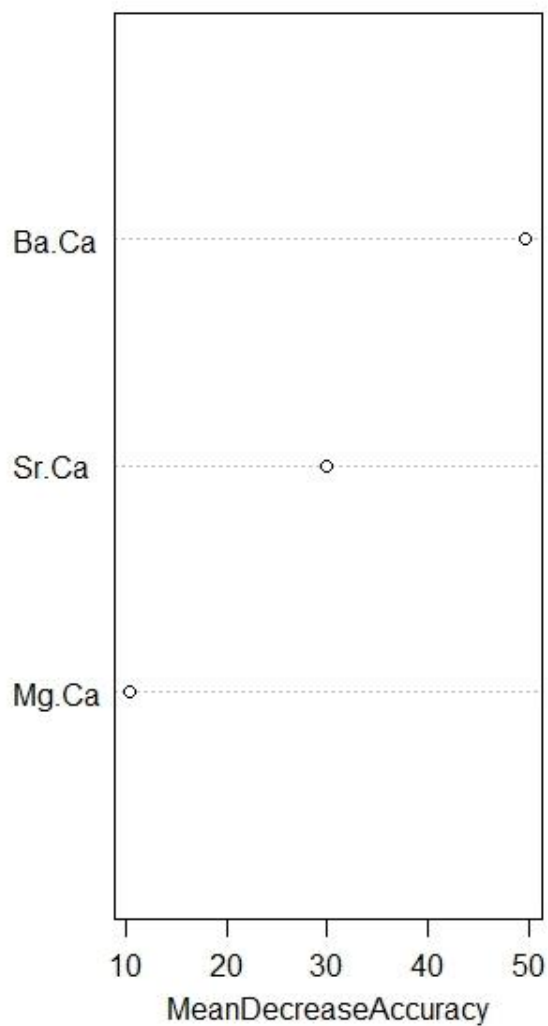


Figure 5. Variable importance plot for predictor variables from the random forest model using 2013 June Sucker otolith chemistry data. The mean decrease in accuracy indicate the importance of each predictor variable, variables with high values for mean decrease in accuracy are more important to the classification model.

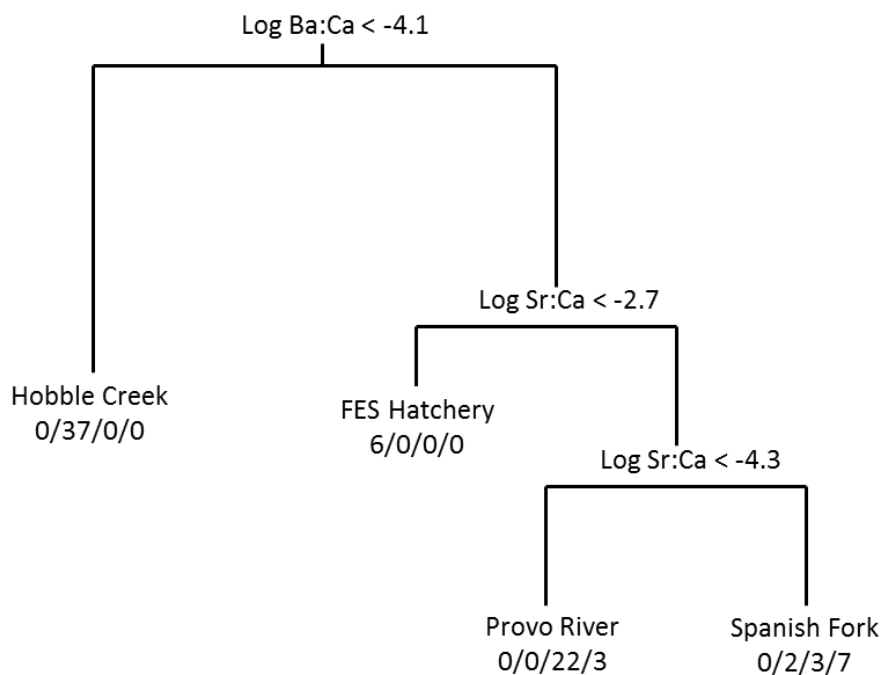


Figure 6. Classification tree of fish to respective tributary based on concentrations of log element:Ca ratios in June Sucker sagittal otoliths. The data are the average otolith element:Ca ratios (Sr:Ca, Ba:Ca, and Mg:Ca) from LA-ICPMS of the outer edge of the otolith. Data were normalized, and measurements that did not meet the limit of detection or were not measured precisely were removed. Each split in the tree is a condition for the classification. The slashes at the nodes represent each tributary (FES Hatchery/Hobble Creek/Provo River/Spanish Fork), and the numbers represent how many individual fish were classified to the tributary at that node. The classification tree has an overall classification rate of 88.9%, correctly classifying 100% of the FES hatchery fish, 94.8% of the Hobble Creek fish, 84.6% of the Provo River fish, and 70% of the Spanish Fork fish.

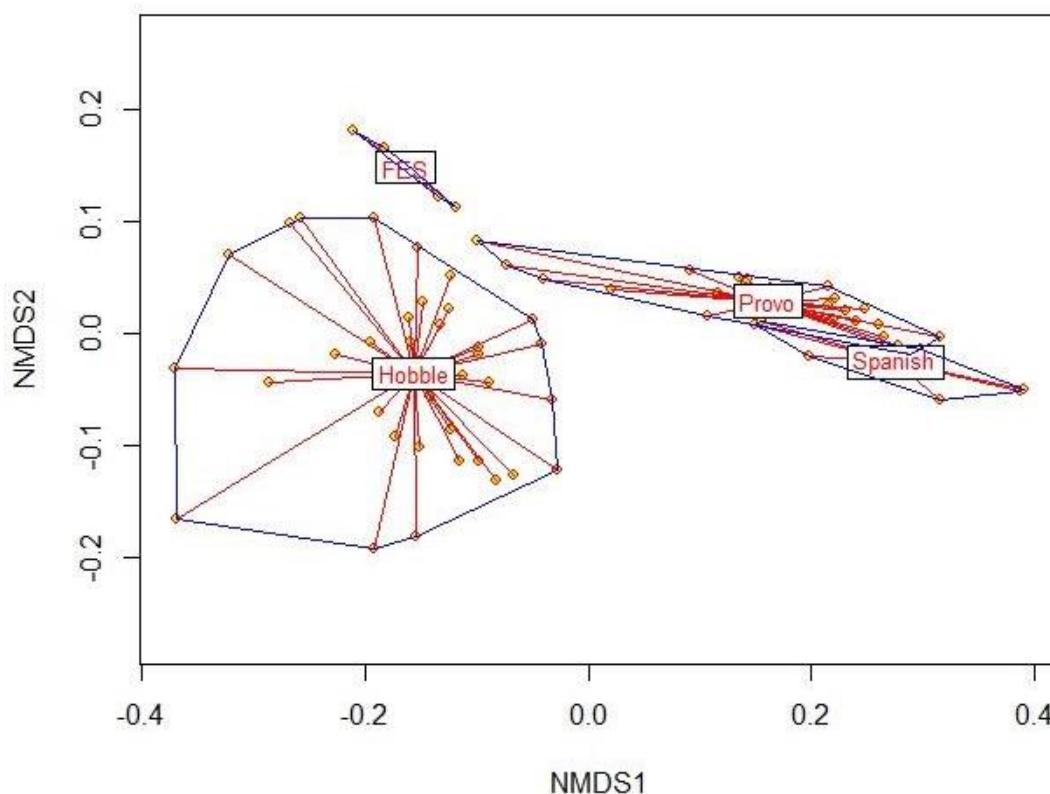


Figure 7. Non-metric multidimensional (NMDS) scaling ordination of June Sucker sagittal otolith microchemistry using predictor variables: Sr:Ca, Ba:Ca, and Mg:Ca. The dots indicate individual June Sucker otolith samples element:Ca ratios plotted in ordination space, fish from streams with similar otolith chemistry are plotted closer together.

hatchery vs. wild) of new individuals recruited into the adult population. My analysis of water chemistry and otolith chemistry revealed that otolith chemistry can be used to establish natal origin of the June Sucker, as well as to provide managers with a tool to determine if natural recruitment is occurring in the Utah Lake basin. The primary goal of this study was to determine if otolith microchemistry could be used as a tool to establish natal origin, to evaluate whether natural recruitment results after habitat restoration. When using otolith microchemistry as a technique to determine the natal origin, the chemistry of the tributaries must be statistically significantly different from

one another, it must be temporally stable, and the differences in water chemistry must be reflected in the otolith chemistry.

There was significant variation in water chemistry among the three major tributaries to Utah Lake, yet stream chemistry did not vary significantly for individual streams throughout the duration of the study. The variation observed among the tributary water chemistry was likely due to the heterogeneity in the underlying geology of the watersheds. In addition anthropogenic impacts of different land uses (e.g., urban and agricultural) could influence the concentration of trace metals within the tributaries. Streams can demonstrate specific chemical signatures due to different geological characteristics, weathering processes, and groundwater recharge among respective drainages (Pangle et al. 2010). Streams within close geographic proximity can exhibit distinguishable variations in water chemistry over small spatial scales (Veinott and Porter 2004). These chemical differences in the water chemistry are directly related to the chemistry found in the otoliths. Accordingly, we observed a strong linear relationship between the element:Ca ratio in the water chemistry and the element:Ca ratio in the otolith chemistry for Sr and Ba, but not for Mg.

To date, Sr and Ba have been recognized as detectable and discriminatory trace elements for use in otolith microchemistry (Ludsin et al. 2006). Otoliths incorporate Sr and Ba into the aragonite in proportion to the Sr and Ba concentrations occur in the ambient water chemistry (Gibson-Reinemer 2009). Magnesium is also frequently used in otolith microchemistry; however, in this study Mg in the otolith did not demonstrate a linear relationship with the water chemistry. Similarly, in a recent study examining the

incorporation of Mg into fish otoliths, Woodcock et al. (2012) also found that Mg concentrations did not change in response to Mg concentrations in the water, suggesting Mg may not be a reliable environmental indicator. In addition, Mg is a major element; therefore, the water chemistry has Mg concentrations several orders of magnitude higher than trace elements Ba and Sr. There may be a limitation to the amount of Mg that can be substituted into the CaCO_3 matrix of the otolith, and this limit could be related to chemical saturation of Mg in the otolith mineral, or a physiological regulation of how much Mg is incorporated into the otolith. In either case, I hypothesize the dissolved Mg in the water exceeds this limit such that the otolith is not sensitive to fluctuations in water chemistry; therefore no linear relationship between water and otolith chemistry is observed. If the Mg concentrations drop significantly below this 'limit' then it is possible it may be reflected in the otolith.

My classification models were successful in predicating fish origin based on otolith chemistry. Overall the otolith element:Ca classification models performed relatively well, with the highest classification model accuracy of 91.4% from the out-of-bag random forest, and the lowest classification accuracy of 88.9% from the ten-fold cross-validated classification tree. For comparison, classification accuracies in other somewhat similar studies have ranged from 100% to 67% for juvenile Yellow Perch (*Perca flavescens*) in Lake Erie tributaries (Pangle et al. 2010); and 97% to 89% reclassification accuracy for age-0 Alewives (*Alosa pseudoharengus*) and age-0 Blueback Herring (*A. aestivalis*) in the Delaware and Rappahannock rivers (Turner and Limburg 2014). While most otolith chemistry studies use linear or quadratic discriminant

analyses for classification, I chose to use the classification trees and random forest to model otolith chemistry, because the techniques do not require that assumptions of normality or homoscedascicity be met and have higher classification accuracy compared to other classification models (Cutler et al. 2007, Carlisle et al. 2009). In addition, are easy to interpret (Cutler et al. 2007). The random forest models build upon the classification trees to improve classification accuracy; they have the capability of modeling complex interactions, produce variable importance plots, and are stable to small perturbations of the data (Cutler et al. 2007). In my analysis both the classification tree and random forest indicated similar results. In addition, the NMDS ordination visually supported the findings of both classification models, demonstrating the complete separation of the hatchery fish (FES) and Hobbie Creek, and partial overlap between Provo River and Spanish Fork. The overlap in Sr:Ca otolith chemistry between Provo River and Spanish Fork fish may be due to the small sample size (n=10) of fish from Spanish Fork, the lake influence at the downstream cage site, similar underlying geology, or some combination of these factors.

The ambient water Ca concentrations may also contribute to the observed overlap in otolith chemistry for Spanish Fork and Provo River fish. Calcium concentrations in the water have a large effect on elemental uptake (Campana 1999). In freshwater fish, the uptake of metals from the gills generally decreases as the ambient concentration of Ca in water increases (Mayer et al. 1994, Campana 1999). When ambient Ca concentration is low, a greater proportion of Ca and trace metals will be absorbed through the gills (Mayer et al. 1994, Campana 1999). This relationship may

also explain the high Ba:Ca concentrations observed in the Hobble Creek fish (Figure A.2). Hobble Creek has significantly lower ambient Ca concentration than the other tributaries (Table A.3); it is the smallest of the three watersheds, and primarily drains sedimentary rock and Lake Bonneville clay deposits. The high Ba:Ca ratio in Hobble Creek is likely due to the low ambient Ca concentration, not a high Ba concentration (Table A.3). The Provo River watershed has the largest area, and primarily consists of a combination of sandstone, limestone, alluvium, and volcanic rock types; it eventually flows through Lake Bonneville deposits (clays) as it nears Utah Lake. Similarly, the Spanish Fork River, the second largest of the watersheds, consists of a combination of sedimentary and volcanic rock types along its length, and Lake deposits as it drains into Utah Lake (Figure A.3). The Provo River and Spanish Fork have a relatively higher ambient Ca concentration than Hobble Creek (Table A.3).

The elemental concentrations in river water is determined by a combination of the bedrock and sediment composition, chemical weathering, and groundwater recharge from limestone, sedimentary evaporite, and silicate rock aquifers (Palmer and Edmond 1992). Limestones are primarily composed of calcium carbonate, have relatively high Sr concentration, and are more susceptible to chemical weathering than silicate rock types (Palmer and Edmond 1992). Evaporite deposits are also easily weathered compared to silicate bedrock and have a relatively high Sr content (Palmer and Edmond 1992). Silicate bedrock, in addition to being resistant to weathering, contains relatively low Sr contents compared to limestones and evaporites (Palmer and Edmond 1992). The smaller drainage area of Hobble Creek watershed may have less

time for groundwater recharge and shorter paths through carbonate bedrock, and weathering processes, which may contribute to its lower Ca concentrations. The Provo River watershed and Spanish Fork watersheds are larger, have more groundwater-rock and weathering interactions, and also consist of bedrock that is relatively higher in Ca concentration (e.g., limestones) than Hobbie Creek.

Despite some remaining uncertainties in the underlying mechanisms driving the water chemistry and elemental uptake from the fish, the models are capable of accurately classifying fish from Utah Lake tributaries and the FES hatchery with relatively low model error (9%-11%). This discrimination is important in that it allows me to predict whether a fish is of wild origin, i.e., if the natal signature does not match the FES hatchery signature. Furthermore, the classification models were able to predict which tributary an individual came from based on otolith chemistry, with relatively high accuracy (89% and 91%). Identifying where individual fish came from based on otolith chemistry provides a useful tool, potentially increasing the ability to determine if a fish came from a restored stream.

As the June Sucker is endangered and research mortality is discouraged, I was unable to collect naturally spawned fish and thus used hatchery-reared fish as a surrogate. Consequently, I could not identify natal origin directly from the otolith core. However, larval otoliths begin to incorporate elements during egg incubation (Campana and Neilson 1985); therefore, I assumed the core of naturally spawned June Sucker otoliths reflect the elemental signature of the natal tributaries. In addition, the downstream site location of the cages accounts for any lake influence that may be

observed in the fish otolith from spending time near the mouth of the river in potential rearing habitat. The water chemistry revealed that Utah Lake had significantly higher Sr:Ca concentrations than the spawning tributaries and the FES hatchery (Table A.2); it is possible this difference may be observed in otolith chemistry taken from a transect across the otolith core to outer edge. In future work or other studies, the trajectory from the core to the outer edge from a wild fish could provide a record of the natal origin, tributary residence time, and possibly spawning events, allowing for a better understanding of life history and habitat use across the fish's life span (e.g., Turner and Limburg 2014).

Future research may benefit from focusing on sampling otoliths from new adult fish of unknown origin, and using the classification models developed to predict if the fish is of wild or hatchery origin. Investigating the use of hard-part chemistry in fin-rays as non-lethal means for determining natal origin in the endangered June sucker may also be a possible avenue that would be beneficial for future research (Wells et al. 2003; Wolff et al. 2013). In addition, water samples should continue to be collected to monitor the inter-annual temporal stability of the water chemistry.

The results from this study will allow managers to track progress of June sucker recovery, by providing a tool for managers to determine if a fish is of hatchery or wild origin, and allowing determination of whether or not natural recruitment is occurring into the adult population. In addition, the classification model will aid in evaluating the effectiveness of habitat restoration across different tributaries of varying restoration progress and state of degradation, by predicting which tributaries wild fish were

spawned in. Similarly these results will help prioritize and direct future restoration plans. Prior to this research, primarily LDA and QDA have been the primary analytical tools used in otolith microchemistry studies; however, my study shows the strength and applicability of classification tree and random forest models for this type of data. In sum we have demonstrated that sufficient differences exist in water chemistry among tributaries, and that June Sucker otoliths reflect the microchemistry of the surrounding ambient water, that knowledge can be used to bridge the gaps remaining in understanding drivers of June sucker recruitment dynamics and prioritizing tributary restoration efforts.

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APPENDIX

Table A.1. Average total length (\pm SD; mm) of June Sucker's at the beginning and end of the cage experiment from the two site locations (upstream and downstream) in the three tributaries (Provo River, Hobble Creek, and Spanish Fork) and the FES hatchery. Included is the number of fish from the cages at each site (N), the average temperature ($^{\circ}$ C; \pm SD) and dissolved oxygen (Avg DO; \pm SD) taken at time of water samples, and the number of fish otoliths from each site that were analysed using LA-ICPMS (otoliths LA).

Stream	Site	Start length	End length	N	Average temp	Average DO	otoliths (n)
Provo River	upstream	17.7 \pm 1.5	31.2 \pm 6.2	5	19.6 \pm 2.4	4.9 \pm 2.7	5
Hobble Creek	upstream	17.7 \pm 1.5	28.1 \pm 4.6	30	18.5 \pm 1.1	3.3 \pm 2.3	18
Spanish Fork	downstream	17.7 \pm 1.5	17.1 \pm 3.7	10	23.92 \pm 3.7	2.6 \pm 2.0	10
FES hatchery	hatchery	17.7 \pm 1.5	31.6 \pm 4.7	6			6
Provo River	downstream	17.7 \pm 1.5	26.4 \pm 3.5	30	22.5 \pm 2.2	2.9 \pm 1.8	21
Hobble Creek	downstream	17.7 \pm 1.5	28.3 \pm 4.9	36	20.3 \pm 1.8	3.4 \pm 2.3	20

Table A.2. June Sucker otolith element:Ca concentrations. The values reported are averages of the normalized, background corrected data from the laser ablation transect across the outer perimeter of the otolith.

ID	Stream	Site	LogMg:Ca	LogSr:Ca	LogBa:Ca
F3	FES Hatchery	F	-4.56	-2.75	-5.05
F17	FES Hatchery	F	-4.36	-2.75	-4.99
F20	FES Hatchery	F	-4.56	-2.73	-4.94
F14	FES Hatchery	F	-4.48	-2.77	-4.86
F13	FES Hatchery	F	-4.82	-2.72	-4.97
F12	FES Hatchery	F	-4.57	-2.75	-5.06
HL1	Hobble Creek	HL	-4.45	-2.75	-3.47
HL3	Hobble Creek	HL	-4.56	-2.75	-3.17
HL6	Hobble Creek	HL	-4.37	-2.79	-3.48
HL9	Hobble Creek	HL	-4.63	-2.73	-4.02
HL10	Hobble Creek	HL	-4.64	-2.69	-3.67
HL14	Hobble Creek	HL	-4.99	-2.71	-3.49
HL20	Hobble Creek	HL	-4.43	-2.70	-3.45
HL21	Hobble Creek	HL	-4.46	-2.69	-3.80
HL33	Hobble Creek	HL	-4.61	-2.70	-3.58
HL36	Hobble Creek	HL	-4.32	-2.72	-3.63
HL28	Hobble Creek	HL	-4.52	-2.68	-3.69
HL29	Hobble Creek	HL	-4.34	-2.70	-3.45
HL39	Hobble Creek	HL	-4.55	-2.71	-3.68
HL24	Hobble Creek	HL	-4.49	-2.71	-3.80
HL30	Hobble Creek	HL	-4.51	-2.67	-3.44
HL11	Hobble Creek	HL	-4.71	-2.72	-3.82
HL35	Hobble Creek	HL	-4.35	-2.71	-3.66
HL25	Hobble Creek	HL	-4.60	-2.68	-4.07
HL17	Hobble Creek	HL	-4.50	-2.71	-4.03
HL12	Hobble Creek	HL	-4.49	-2.67	-3.94
HU12	Hobble Creek	HU	-4.48	-2.71	-3.88
HU9	Hobble Creek	HU	-4.38	-2.68	-3.48
HU25	Hobble Creek	HU	-4.56	-2.79	-3.72
HU24	Hobble Creek	HU	-4.40	-2.70	-3.23
HU22	Hobble Creek	HU	-4.44	-2.70	-3.61
HU21	Hobble Creek	HU	-4.53	-2.66	-3.46
HU20	Hobble Creek	HU	-4.38	-2.68	-3.46
HU19	Hobble Creek	HU	-4.46	-2.69	-3.28
HU31	Hobble Creek	HU	-4.60	-2.74	-3.56
HU30	Hobble Creek	HU	-4.66	-2.69	-3.77

Table A.2. June Sucker otolith element:Ca concentrations (cont.)

ID	Stream	Site	LogMg:Ca	LogSr:Ca	LogBa:Ca
HU29	Hobble Creek	HU	-4.52	-2.69	-3.52
HU28	Hobble Creek	HU	-4.48	-2.65	-3.50
HU27	Hobble Creek	HU	-4.66	-2.72	-3.75
HU26	Hobble Creek	HU	-4.95	-2.66	-3.71
HU7	Hobble Creek	HU	-4.48	-2.70	-3.70
HU34	Hobble Creek	HU	-4.40	-2.77	-3.86
HU33	Hobble Creek	HU	-4.51	-2.77	-3.88
HU32	Hobble Creek	HU	-4.24	-2.74	-4.03
PL3	Provo River	PL	-4.65	-2.59	-4.40
PL2	Provo River	PL	-4.58	-2.54	-4.35
PL1	Provo River	PL	-5.02	-2.57	-4.49
PL14	Provo River	PL	-4.88	-2.59	-4.56
PL13	Provo River	PL	-4.90	-2.61	-4.61
PL15	Provo River	PL	-4.68	-2.61	-4.48
PL12	Provo River	PL	-5.09	-2.60	-4.55
PL9	Provo River	PL	-4.89	-2.57	-4.71
PL8	Provo River	PL	-4.70	-2.55	-4.56
PL7	Provo River	PL	-4.53	-2.61	-4.27
PL6	Provo River	PL	-4.76	-2.56	-4.35
PL5	Provo River	PL	-4.48	-2.56	-4.54
PL17	Provo River	PL	-4.59	-2.55	-4.43
PL18	Provo River	PL	-4.58	-2.52	-4.49
PL21	Provo River	PL	-4.60	-2.56	-4.40
PL25	Provo River	PL	-4.63	-2.53	-4.27
PL26	Provo River	PL	-5.04	-2.54	-4.25
PL27	Provo River	PL	-4.63	-2.57	-4.56
PL28	Provo River	PL	-4.65	-2.59	-4.69
PL29	Provo River	PL	-4.68	-2.60	-4.65
PL30	Provo River	PL	-4.59	-2.60	-4.42
PU2	Provo River	PU	-4.57	-2.71	-4.58
PU1	Provo River	PU	-4.37	-2.70	-4.57
PU5	Provo River	PU	-4.90	-2.63	-4.72
PU4	Provo River	PU	-4.16	-2.66	-4.43
PU3	Provo River	PU	-4.31	-2.68	-4.54
SL10	Spanish Fork	SL	-4.49	-2.59	-4.23
SL9	Spanish Fork	SL	-4.46	-2.57	-4.07

Table A.2. June Sucker otolith element:Ca concentrations (cont.)

ID	Stream	Site	LogMg:Ca	LogSr:Ca	LogBa:Ca
SL8	Spanish Fork	SL	-4.40	-2.54	-4.29
SL7	Spanish Fork	SL	-4.45	-2.52	-4.27
SL6	Spanish Fork	SL	-4.58	-2.59	-4.24
SL5	Spanish Fork	SL	-4.98	-2.49	-4.19
SL4	Spanish Fork	SL	-4.44	-2.48	-4.25
SL3	Spanish Fork	SL	-4.60	-2.53	-4.31
SL2	Spanish Fork	SL	-4.48	-2.51	-4.10
SL1	Spanish Fork	SL	-4.48	-2.54	-4.22

Table A.3. Water chemistry collected at the three main spawning tributaries of Utah Lake; Provo River, Hobble Creek, and Spanish Fork. Water samples were collected at the upstream site (US) and the downstream site (DS) at each tributary from June through the end of August in 2013. Reported values are the averages of the two 60 mL replicate samples.

Stream	Site	Date	Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu
Hobble Creek	DS	6/5/2013	0.03	5.04	0.11	0.32	9.83	7.28	0.11	0.25	0.28
Hobble Creek	DS	7/10/2013	0.01	5.35	0.07	0.40	11.45	18.37	0.10	0.24	0.21
Hobble Creek	DS	7/11/2013	0.01	7.68	0.40	0.33	12.86	14.91	0.11	0.29	0.22
Hobble Creek	DS	8/15/2013	0.02	5.02	0.15	0.30	13.89	15.60	0.11	0.26	0.36
Hobble Creek	DS	8/30/2013	0.02	5.90	0.21	0.28	9.29	9.19	0.10	0.20	0.16
Hobble Creek	US	8/15/2013	0.01	4.47	0.02	0.27	17.16	13.92	0.11	0.23	0.17
Hobble Creek	US	8/30/2013	0.01	6.86	0.18	0.28	11.95	8.37	0.10	0.20	0.19
Provo River	DS	7/10/2013	0.01	2.80	0.89	0.31	22.96	19.48	0.09	0.38	0.52
Provo River	DS	8/14/2013	0.02	4.50	1.32	0.32	23.21	25.02	0.07	0.48	0.40
Provo River	DS	8/30/2013	0.00	1.64	1.43	0.30	28.03	25.63	0.09	0.46	0.58
Provo River	US	6/5/2013	0.01	4.03	0.44	0.45	5.04	8.78	0.05	0.30	0.57
Provo River	US	7/10/2013	0.01	3.12	0.47	0.38	5.88	8.69	0.06	0.31	0.80
Provo River	US	8/14/2013	0.01	2.45	1.09	0.35	4.70	17.04	0.07	0.49	0.79
Provo River	US	8/30/2013	0.00	19.62	0.62	0.34	6.85	13.33	0.07	0.41	1.08
Spanish Fork	DS	7/10/2013	0.01	14.42	1.98	0.32	55.78	16.92	0.17	0.62	0.34
Spanish Fork	DS	8/15/2013	0.02	3.25	0.39	0.22	84.59	16.98	0.14	0.46	0.27
Spanish Fork	US	6/5/2013	0.01	4.84	0.57	0.34	91.73	16.21	0.17	0.50	0.39
Spanish Fork	US	7/10/2013	0.01	4.63	1.02	0.29	72.80	10.13	0.15	0.51	0.29
Spanish Fork	US	8/15/2013	0.01	2.35	0.41	0.24	144.81	36.09	0.17	0.52	0.26
FES Hatchery		8/6/2014	-0.01	16.60	0.89	0.82	0.63	8.08	-0.02	0.92	7.32
Utah Lake		7/2/2014	-0.01	4.54	5.38	0.50	0.56	3.25	0.09	1.01	0.91

Table A.3. Water chemistry (cont.)

Stream	Site	Date	Zn	As	Se	Sr	Cd	Sb	Ba	Tl	Pb	U
Hobble Creek	DS	6/5/2013	22.01	0.88	0.14	210.16	2.96	0.05	60.78	-0.05	-0.03	0.44
Hobble Creek	DS	7/10/2013	80.82	0.85	0.19	223.69	2.76	-0.02	108.04	-0.05	-0.06	0.40
Hobble Creek	DS	7/11/2013	139.97	0.84	0.25	220.81	2.65	0.15	190.75	-0.05	-0.05	0.50
Hobble Creek	DS	8/15/2013	23.22	0.93	0.20	216.53	2.69	0.06	82.69	-0.05	-0.05	0.47
Hobble Creek	DS	8/30/2013	69.33	1.05	0.18	227.84	2.44	-0.01	101.72	-0.05	-0.07	0.43
Hobble Creek	US	8/15/2013	27.75	0.92	0.13	219.78	2.53	0.01	80.15	-0.05	-0.07	0.42
Hobble Creek	US	8/30/2013	116.43	1.03	0.17	224.89	2.59	0.03	83.86	-0.05	-0.08	0.45
Provo River	DS	7/10/2013	26.04	2.98	0.58	398.31	2.46	0.16	108.77	-0.05	-0.03	0.88
Provo River	DS	8/14/2013	208.33	4.06	0.80	521.37	2.66	0.22	117.65	-0.05	-0.05	1.28
Provo River	DS	8/30/2013	16.25	4.35	0.75	479.14	2.54	0.27	100.86	-0.05	-0.05	1.20
Provo River	US	6/5/2013	23.71	1.85	0.56	375.26	3.44	0.15	75.76	-0.03	-0.03	0.85
Provo River	US	7/10/2013	92.96	2.20	0.59	390.51	2.58	0.10	109.15	-0.04	-0.07	0.85
Provo River	US	8/14/2013	87.16	2.04	0.71	499.78	2.89	0.16	119.70	-0.03	-0.06	1.55
Provo River	US	8/30/2013	62.40	2.37	0.78	468.75	2.48	0.14	106.12	-0.04	-0.07	1.15
Spanish Fork	DS	7/10/2013	62.84	3.60	0.52	652.71	2.58	0.12	118.99	-0.05	-0.05	1.26
Spanish Fork	DS	8/15/2013	20.37	1.34	0.41	488.10	2.31	0.05	100.01	-0.05	-0.07	0.81
Spanish Fork	US	6/5/2013	1.66	1.51	0.59	584.87	3.24	0.02	87.74	-0.05	-0.02	1.11
Spanish Fork	US	7/10/2013	172.69	1.76	0.42	569.78	2.69	0.11	121.05	-0.05	-0.05	0.92
Spanish Fork	US	8/15/2013	17.49	1.61	0.35	621.79	2.21	0.01	110.04	-0.06	-0.07	0.81
FES Hatchery		8/6/2014	24.67	0.93	0.07	253.67	2.59	-0.31	68.81	-0.02	0.23	
Utah Lake		7/2/2014	115.64	11.26	1.07	1314.24	3.63	0.47	125.26	-0.03	0.20	

Table A.3. Water chemistry (cont.)

Stream	Site	Date	Na	Mg	K	Ca	Si
Hobble Creek	DS	6/5/2013	6834.26	11819.07	706.2595	1231.855	41013.4
Hobble Creek	DS	7/10/2013	7671.89	13902.78	1814.799	1421.545	44400.49
Hobble Creek	DS	7/11/2013	7604.46	14068.85	1785.743	1312.949	45957.74
Hobble Creek	DS	8/15/2013	7424.19	13045.51	1076.046	1349.882	44815.64
Hobble Creek	DS	8/30/2013	7157.43	12403.03	1153.974	1337.373	44346.64
Hobble Creek	US	8/15/2013	7283.14	12595.79	914.9723	1367.399	43807.42
Hobble Creek	US	8/30/2013	7236.79	12583	1219.58	1324.65	44898.77
Provo River	DS	7/10/2013	16766.83	16773.31	4076.424	3176.804	64911.72
Provo River	DS	8/14/2013	25119.17	23137.72	4870.891	5018.376	77875.59
Provo River	DS	8/30/2013	21164.32	20362.2	5073.436	4235.779	77221.31
Provo River	US	6/5/2013	15477.63	15741.45	2963.294	2584.356	63752.89
Provo River	US	7/10/2013	16778.96	16322.36	3314.52	2734.44	65955.85
Provo River	US	8/14/2013	25384.12	21895.89	4082.205	3888.468	85215.47
Provo River	US	8/30/2013	20631.34	19012.82	3752.15	3194.799	79900.36
Spanish Fork	DS	7/10/2013	82736.86	35720.48	6205.046	5298.997	60605.03
Spanish Fork	DS	8/15/2013	45147.65	25369.01	2333.33	3047.856	60768.78
Spanish Fork	US	6/5/2013	63980.75	31476.18	3890.23	3396.718	65067.88
Spanish Fork	US	7/10/2013	62547.53	30940.84	5539.13	3595.98	65487.5
Spanish Fork	US	8/15/2013	81528.55	33782.62	4318.055	3763.058	68642.1
FES Hatchery		8/6/2014	2627.38	2220.829	328.2586	5023.625	
Utah Lake		7/2/2014	22466.45	6795.053	1980.267	6608.588	

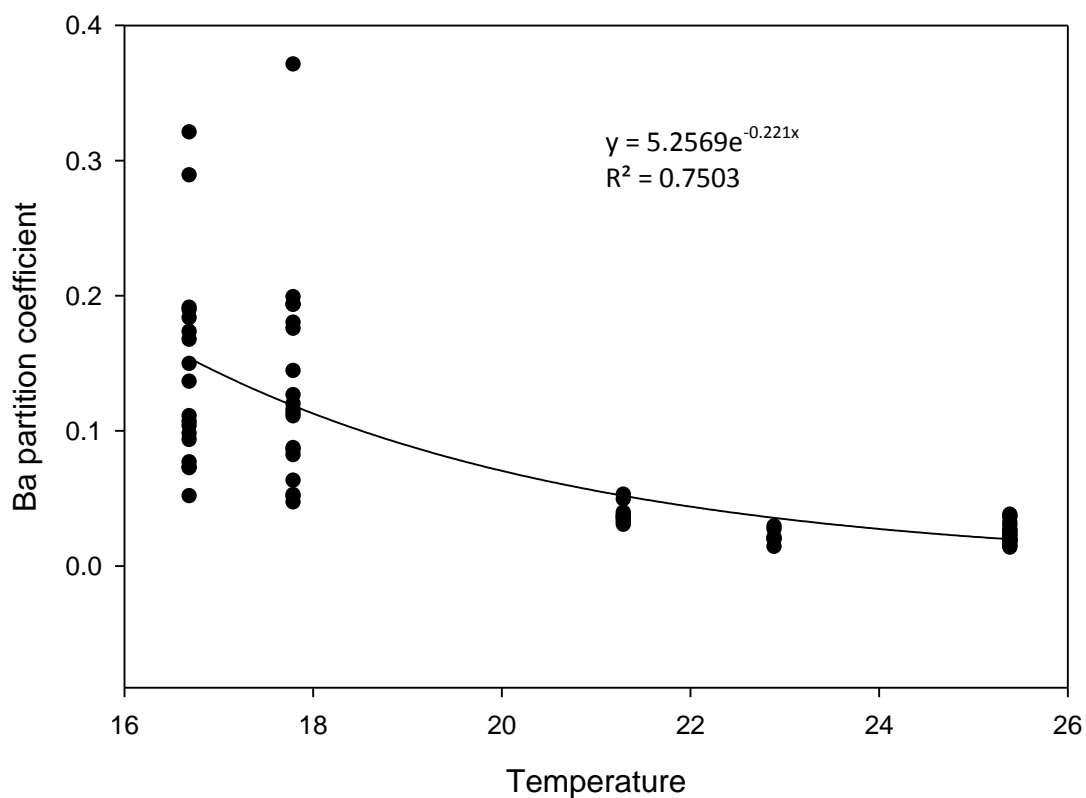


Figure A.1. Exponential model for the June Sucker Ba partition coefficient as a function of stream temperature (°C). Temperature measurement was taken from each tributary study site location (upstream and downstream) on the August 15th water sampling date. The Ba partition coefficients are calculated as the Ba (ppm) of the water chemistry (from August 15th 2013) divided by the Ba concentration (ppm) from the June Sucker otolith chemistry.

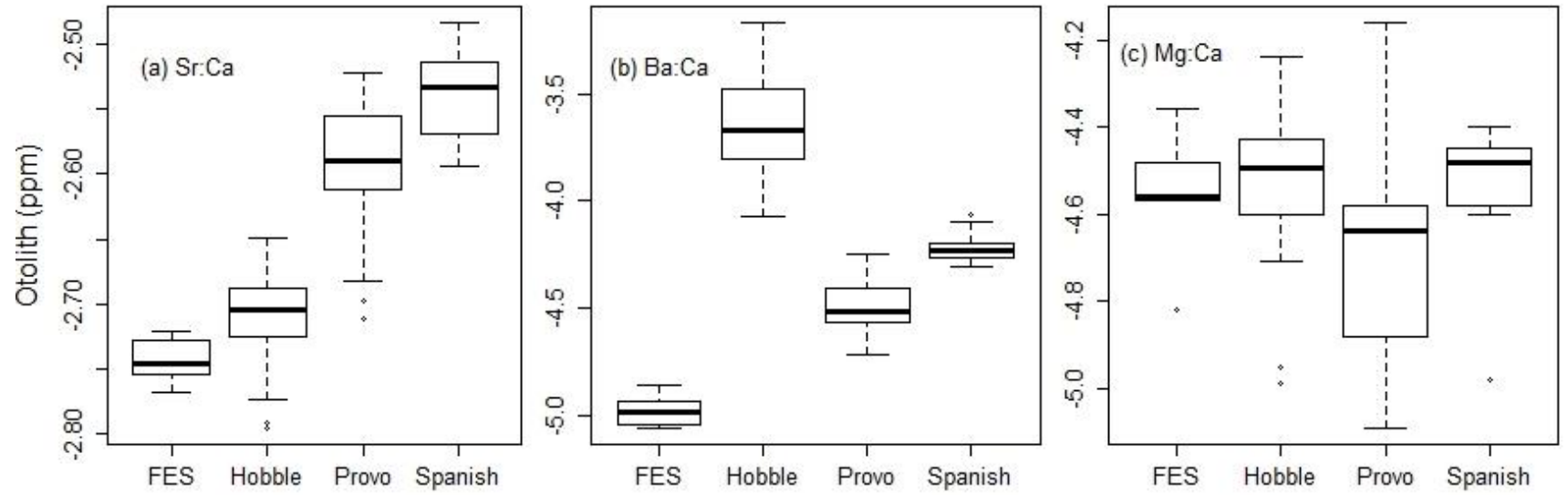


Figure A.2. Box plots of fish otolith elemental concentrations by site, all data were log transformed. Horizontal lines in the boxes represent the median, the top and bottom of the box represent the first and third quintile, the whiskers are the 5th and 95th percentile of the median, and the circles are outliers. The classification models use the combination of elements, there may be overlap in one element but not another.

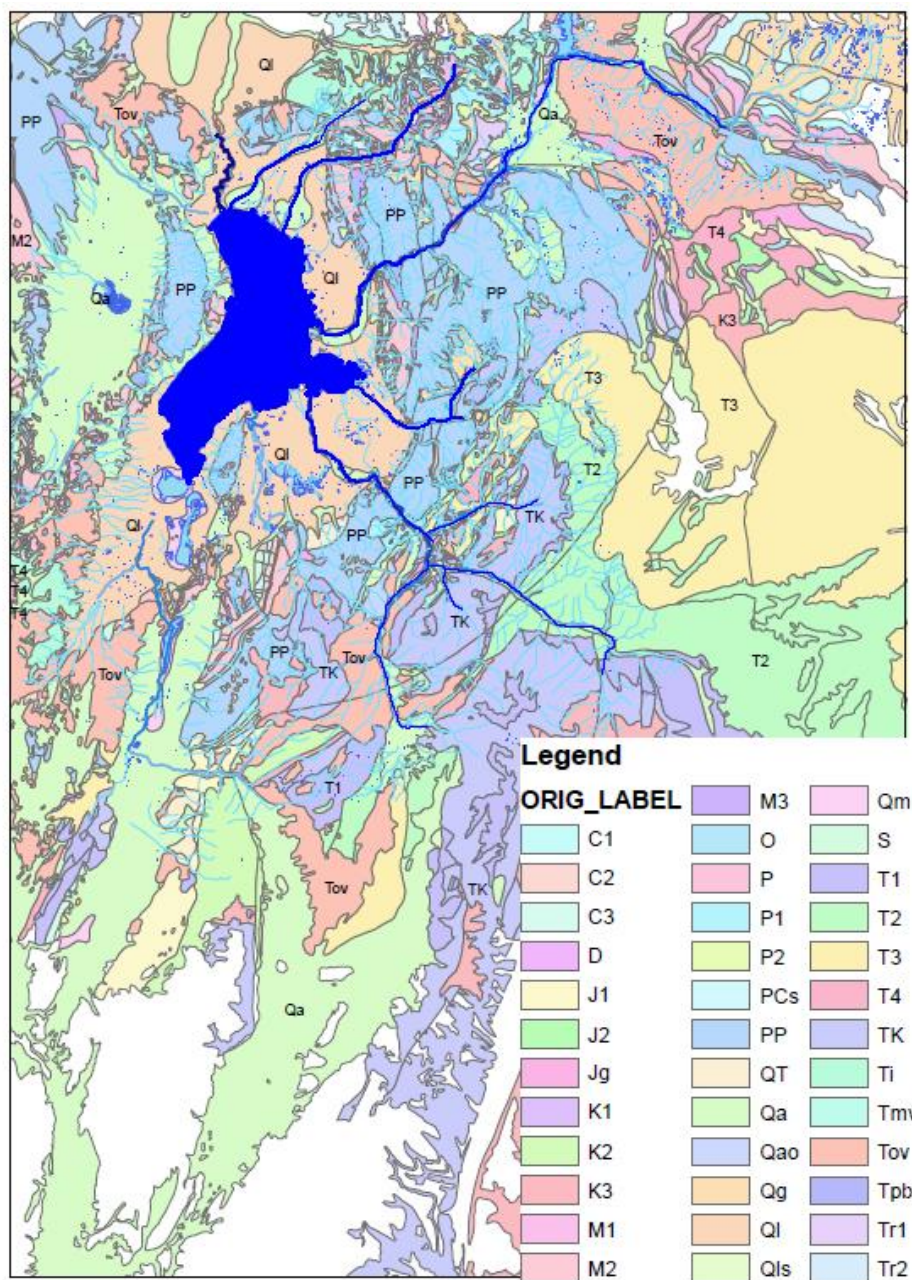


Figure A.3. Geologic map of Utah Lake Utah, and its tributaries. The main-stem of the tributaries are outlined in blue as Spring Creek (top), American Fork Provo River, Hobbie Creek, and Spanish Fork (bottom). The river outlined in dark blue, above Spring Creek, is Utah Lake's output the Jordan River, which flows into the Great Salt Lake. The legend on the right represents the different types of geology, and the legend key is continued on the next page.

LABEL	UNIT_NAME	UNIT_AGE	STRAT_UNIT	ROCKTYPE1
C1	Cambrian quartzite in central Utah	Early to Middle Cambrian	Tintic Quartzite	arenite
C2	Middle Cambrian shale and carbonate rocks in central Utah	Middle Cambrian	Ophir Formation, Maxwell, Herkimer, and Teutonic Limestones, and Cole Canyon, bluebird, and Dagmar Dolomites	dolostone (dolomite)
C3	Upper Cambrian carbonate rocks in central Utah	Middle Cambrian to Early	Opex Formation, Ajax Dolomite, and Maxwell Limestone	dolostone (dolomite)
D	Devonian sedimentary rocks in central Utah	Devonian	Victoria Formation and Pinyon Peak Limestone	limestone
J1	Jurassic (1) sedimentary rocks in central Utah	Middle Jurassic	Twist Gulch and Carmel Formations, Arapien Shale, Twin Creek Limestone, and Entrada Sandstone	mudstone
J2	Jurassic (2) sedimentary rocks in Uinta Mountains-Uinta Basin region	Late Jurassic	Morrison Formation	shale
Jg	Triassic and Jurassic sedimentary rocks in central Utah	Early Jurassic	Glen Canyon Sandstone	sandstone
K1	Cretaceous (1) sedimentary rocks in Uinta Mountains-Uinta Basin region	Cretaceous	Dakota and Cedar Mountain Formations	sandstone
K2	Cretaceous (2) sedimentary rocks in central Utah	Cretaceous	Indianola Group	sandstone
K3	Cretaceous (3) sedimentary rocks in central Utah	Late Cretaceous	Price River and South Flat Formations	sandstone
M1	Mississippian (1) carbonate rocks in central Utah	Late Devonian to Mississippian	Gardison Limestone and Fitchville Formation	limestone
M2	Mississippian (2) sedimentary rocks in central Utah	Mississippian	Humburg Formation and Great Blue and Deseret Limestones	limestone
M3	Mississippian (3) sedimentary rocks in central Utah	Mississippian	Manning Canyon Shale	shale
O	Ordovician sedimentary rocks in central Utah	Ordovician	Opohonga Limestone and Fish Haven Dolomite	limestone
P1	Permian (1) sedimentary rocks in central Utah	Early Permian	Diamond Creek Sandstone and Kirkman Limestone	sandstone
P2	Permian (2) sedimentary rocks in central Utah	Permian	Park City and Phosphoria Formations	limestone
P	Pennsylvanian sedimentary rocks in central Utah	Pennsylvanian	Morgan Formation and Round Valley Limestone	limestone
PCs	Younger Precambrian metamorphic rocks in central Utah	Proterozoic Z	Mutual, Inkom, Papoose Creek, and Pocatello Formations, Caddy Canyon Quartzite, and Black Rock Canyon Limestone	sandstone
playa	playa	Holocene		playa
PP	Pennsylvanian and Permian sedimentary rocks in central Utah	Pennsylvanian to Permian	Oquirrh Group, including Bridal Veil Limestone	sandstone
Qa	Quaternary alluvium and colluvium	Quaternary		alluvium
Qao	Older Quaternary alluvial deposits	Quaternary		alluvium
Qg	Quaternary glacial deposits	Quaternary		glacial drift
Qj	Quaternary Lake Bonneville deposits	Quaternary		clay or mud
Qjs	Quaternary landslides	Quaternary		landslide
Qm	Quaternary marshes	Holocene		biogenic
QT	Pliocene and Quaternary alluvial material	Pliocene to Quaternary	Axtell and Harkers Formations	alluvium
S	Silurian carbonate rocks in central Utah	Late Ordovician to Silurian	Bluebell Dolomite	dolostone (dolomite)
T1	Tertiary (1) sedimentary rocks in central Utah	Late Paleocene to Early Eocene	Flagstaff Limestone and Canyon Range Formation	limestone
T2	Tertiary (2) sedimentary rocks in Uinta Mountains-Uinta Basin region	Eocene	Green River Formation	shale
T3	Tertiary (3) sedimentary rocks in Uinta Mountains-Uinta Basin region	Middle Eocene to Early Oligocene	Uinta, Bridger, and Duchesne River Formations	sandstone
T4	Tertiary (4) sedimentary rocks in central Utah	Middle Miocene to Late Pliocene	Salt Lake Formation	medium-grained mixed

Figure A.3. Legend Key to the geologic map of Utah Lake, Utah, and its tributaries.